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Original Article

Up-regulation of ICAM-1/VCAM-1 promotes the progression of periodontitis by activating the NF-κB pathway

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Abstract: Periodontitis remains to be one of the most common oral diseases, and brings huge damage to people's life. Inflammations play significant roles in the biology of periodontitis. This study was aimed to investigate the highly expressed proteins of ICAM-1 and VCAM-1 in the development of periodontitis at the inflammatory level and to reveal its possible mechanism. Expression of the targets in the inflamed gingiva-isolated periodontal lymphocytes and normal lymphocytes was measured. The effects of ICAM-1 and VCAM-1 expression on periodontal lymphocytes apoptosis were analyzed. Furthermore, the cell apoptosis- and signal pathway-related protein expression were also analyzed. Compared to the normal lymphocytes, ICAM-1 and VCAM-1 were highly expressed in periodontal lymphocytes. When cells were treated with the ICAM-1/VCAM-1 antibody, the cell apoptosis was enhanced compared to the controls and this effect was more apparent when cells were treated with the two antibodies. Besides, Bcl-2 and Bcl-xs expression was both significantly increased by the suppressed ICAM-1/VCAM-1, whereas the p-IKBα and p-IKKα/β levels were significantly decreased. Similarly, the decreased effects of the suppressed ICAM-1/VCAM-1 on the former proteins expression were more apparent when cells were treated with the two antibodies. Taken together, our study revealed that the overexpressed ICAM-1/VCAM-1 may contribute to the development and progression of periodontitis through inhibiting apoptosis and activating the NF-κB signal pathway.

Keywords: Periodontitis, ICAM-1, VCAM-1, cell apoptosis, NF-κB pathway

Introduction

Periodontitis remains to be one of the most common infectious diseases [1], which is characterized by the gum swelling and bleeding, alveolar bone resorption, periodontal pocket formation, and tooth mobility [2]. Previous evidence refers that periodontitis can bring huge damages on periodontium, as a result of the interaction between the toxic ingredients of periodontal pathogens and the defense mechanism of the specify host [3]. Traditional methods such as clean, drug, surgery, fixed loose tooth, and systemic treatment have obtained certain achievement in the treatment of periodontitis [4, 5], but the results still remain unsatisfactory due to the complicate pathogen mechanism. Hence, to explore the deep pathogen mechanism at the molecular level will be benefit for the treatment of periodontitis.

Studies have revealed that various kinds of factors including diabetes, genetic factors, and microorganisms introduction are the major pathogens in contributing to the development and progression of periodontitis [6-8]. Recent studies have referred that biological processes including cell apoptosis are involved in the biology development of periodontitis [9]. Apoptosis is closely associated with the biology mechanism of diseases such as tumor, autoimmune diseases, and microbial infection diseases [10, 11]. In addition, the metabolites produced by pathogenic bacteria restrained the apoptosis of leukocytes, and then resulted in the inflammatory reactions and the development of periodontitis [12].

Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) are two immune cells that play pivotal roles in leu-

Table 1. Primers used for targets amplification in this study

Target	Primer	Sequence (5'-3')
GAPDH	Sense	GGGTGGAGCCAAACGGGTC
	Antisense	GGAGTTGCTGTTGAAGTCGCA
Bcl-2	Sense	TTGTGGCCTTCTTTGAGTTCGGTG
	Antisense	GGTGCCGGTTCAGGTACTCAGTCA
Bcl-xs	Sense	AGTCAGTTTAGTGATGTC
	Antisense	GGATGTTAGATCACTGAA
ICAM-1	Sense	TGACCGTGAATGTGCTCTCC
	Antisense	TTCCGCTGGCGGTTATAGAG
VCAM-1	Sense	AAGATGGTCGTGATCCTTGG
	Antisense	GGTGCTGCAAGTCAATGAGA

kocytes cell-cell recognition, as well as mediate the signal transduction [13, 14]. Accumulating studies have reported that ICAM-1 is associated with the development of periodontitis [15, 16], as well as VCAM-1 in the development of periodontitis [17]. Additionally, Okada et al reported that the up-regulated ICAM-1/VCAM-1 is correlated to the pathogenesis of allergic keratopathy [18]. Although many studies have revealed the pivotal roles of ICAM-1 and VCAM-1 in the development of periodontitis, yet, the roles of ICAM-1/VCAM-1 in the inflammatory pathogenesis of periodontitis have not been fully reported.

In this study, we investigated the possible effects of the expression of ICAM-1 and VCAM-1 on the development of periodontitis using the inflamed gingiva-isolated periodontal lymphocytes. A variety of experiment methods were used to analyze the expression of the two proteins in the periodontitis and normal tissue, as well as the influences of the two proteins on periodontal lymphocytes apoptosis. This study was aimed to investigate the potential roles of ICAM-1 and VCAM-1 in the development and progression of periodontitis and to reveal its possible molecular mechanism of its action.

Materials and methods

Isolation of periodontal lymphocytes

Periodontal lymphocytes were isolated from the inflamed gingiva and normal gingiva tissues as previously described [19]. In brief, the tissue specimen in AIM V medium (Gibco BRL, Grand Island, NY) containing 125 ng/mL of amphotericin B, proteinase inhibitors (1 μg/mL of α2-macroglobulin, 0.1 μg/mL of α1-antitrypsin, 200 μg/mL of EDTA-2Na, 0.5 μg/mL of aprotinin, and 0.5 μg/mL of E-64), and 10 U/mL of heparin sodium was cut into 1-3 mm pieces with surgical scalpels and extruded through a 73.7-μm-pore-size mesh filter with glass rods. Cells were then isolated by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation. The majority of the isolated cells (more than 99%) showed forward and side scatter identical to those of peripheral blood lymphocytes when judged by flow cytometry. These cells were considered as lymphocyte-rich fractions (periodontal lymphocytes).

Real time (RT)-PCR

Total RNA was isolated from cells using TRIzol Reagent (Invitrogen, CA, USA) as previously described [20]. The concentration and purity of isolated RNA were measured with SMA 400 UV-VIS (Merinton, Shanghai, China). Purified RNA at density of 0.5 μg/μL with nuclease-free water was used for the complementary DNA (cDNA) synthesis using reverse transcriptase (iScript™ cDNA Synthesis Kit; Bio-Rad Laboratories). The expression levels of mRNAs were measured by SYBR green-based quantitative RT-PCR (SYBR Green Master mix; Thermo Scientific, Waltham, MA, USA). Melting curve analysis of amplification products was performed at the end of each PCR to confirm that only one product was amplified and detected. Phosphoglyceraldehyde dehydrogenase (GAPDH) was chosen as the internal control. Primers used for targets amplification were shown in **Table 1**.

Flow cytometry

Flow cytometry was used to examine the protein expression of ICAM-1 and VCAM-1 on the cell surface of cultured periodontal lymphocytes as previously described [18]. Briefly, cells were stained with the antibodies against mouse IgG1 (Invitrogen), ICAM-1 (84H10; Immunotech, Marseille, France) or VCAM-1 (1G11; Immunotech), respectively. Then cells were analyzed by FACScan (Becton Dickinson, Franklin Lane, NJ) and then analyzed using the CellQuest software (Becton Dickinson, Mountain View, CA).

Cell apoptosis assay

Cell apoptosis was analyzed with Annexin V-FITC cell apoptosis kit (Invitrogen, USA)

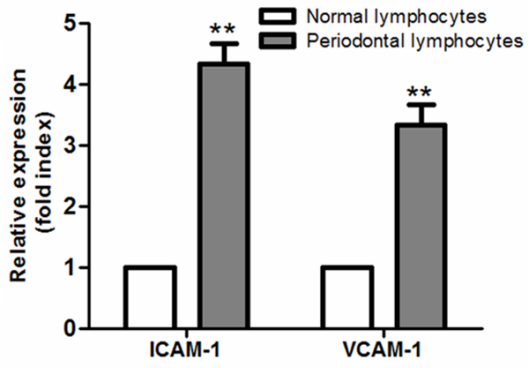

Figure 1. Relative mRNA expression of ICAM-1 and VCAM-1 in lymphocytes. Both the two proteins were highly expressed in the periodontal lymphocytes compared to the normal lymphocytes. **: P<0.01 compared to the normal lymphocytes.

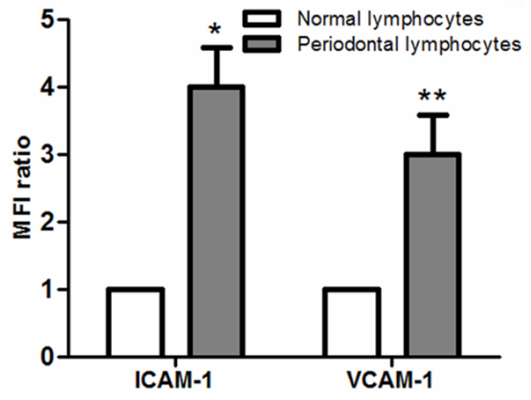

Figure 2. Protein expression of ICAM-1 and VCAM-1 in lymphocytes. Flow cytometry analysis showed that the MFI ratio for ICAM-1 and VCAM-1 were significantly increased compared to the control respectively. *: P<0.05 and **: P<0.01 compared to the normal lymphocytes.

according to manufacturer's protocol. Briefly, after antibody treatment, cells in each group were harvested and then washed using PBS buffer (PH 7.4) for 3 times, and then resuspended in the staining buffer. Then 5 μL of annexin-V-FITC and 5 μL of propidium iodide (PI) were mixed with the cells. After being cultivated at room temperature for 10 min, mixtures were analyzed using the FACScan flow cytometry. Annexin V-positive and propidium iodide-negative cells were considered to be apoptotic cells.

Western blot analysis

Cells that cultured for 48 h in each group were lapped with RIPA assay (radioimmunoprecipitation, Sangon Biotech, China) lysate containing PMSF (phenylmethanesufonyl fluoride, Sigma, USA), and then were centrifuged at 12,000 rpm for 10 min at 4°C. Supertanant was collected for the measurement of protein concentrations using BCA protein assay kit (Pierce, Rochford, IL). For western blot analysis [21], 50 μg protein per cell lysate was subjected onto a 12% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transferred onto the Polyvinylidencefluoride (PVDF) membranes (Millipore). Then the membranes were blocked in the Tris-Buffered Saline Tween (TBST) containing 5% non-fat milk for 1 h at room temperature. The membranes were incubated with rabbit anti-human antibodies (Bcl-2, Bcl-xs, p-IKBα, p-IKKα/β, IKBα, IKKα and IKKβ, 1:100 dilution, Invitrogen) and overnight at 4°C. Then membranes were incubated with horseradish-peroxidase labeled goat anti-rat secondary antibody (1:1000 dilution) at room temperature for 1 h. Finally, the membranes were washed 3 times with 1× TBST buffer for 10 min each. The signals were detected after incubation with a chromogenic substrate using the enhanced chemiluminescence (ECL) method. Additionally, GAPDH served as the internal control.

Statistical analysis

All experiments were repeated 3 times independently. Data are expressed as the mean ± SD. Statistical analysis was performed using Graph Prism 6.0 (GraphPad Prism, San Diego, CA). Significant differences for data among groups were calculated using a one-way analysis of variance (ANOVA) with post-hoc test. The P<0.05 was considered as the statistically significant.

Results

mRNA expression of ICAM-1 and VCAM-1 in the periodontal lymphocytes

The relative mRNA expression for ICAM-1 and VCAM-1 in periodontal lymphocytes was analyzed using RT-PCR (**Figure 1**). Compared to the normal lymphocytes, the relative mRNA expression for targets including ICAM-1 and VCAM-1 was significantly increased (P<0.01), indicating that the protein expression may involve in the pathogen of periodontal.

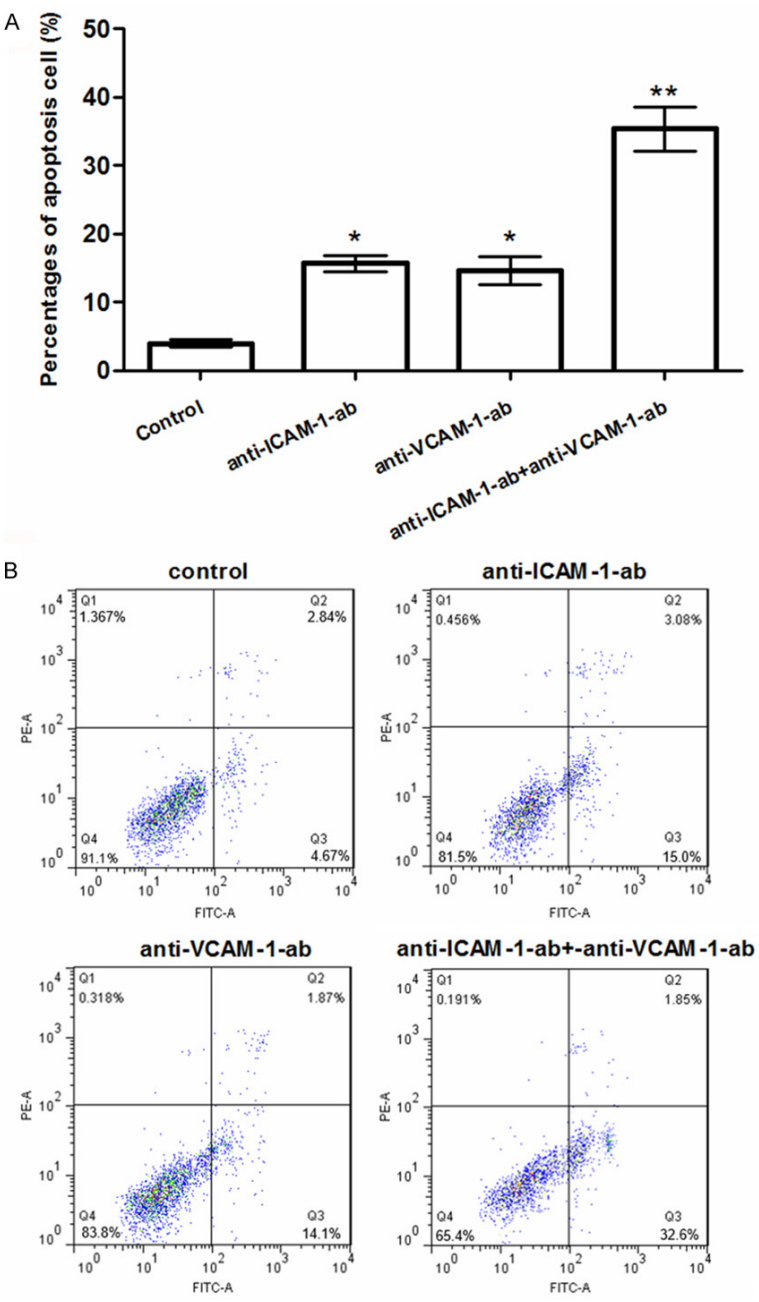

Figure 3. Influences of ICAM-1 and VCAM-1 antibody-treatment on cell apoptosis in lymphocytes. A: The percentage of apoptotic cells were significantly increased by the ICAM-1 or VCAM-1 antibody-treatment, while this effect was more apparent when cells were treated with the two kinds of antibodies. *: P<0.05 and **: P<0.01 compared to the control (periodontal lymphocytes without any antibody treatment); B: Annexin V-FITC showed that the percentage for ICAM-1 antibody-(15.0%) or VCAM-1 antibody-treatment (14.1%) were higher than that in control group (4.57%), whereas this effect was more apparent when cells were treated with the two kinds of antibodies (32.6%).

The abnormal expression of ICAM-1 and VCAM-1 in the periodontal lymphocytes

Accordingly, we analyzed the median fluorescence intensity (MFI) ratio for the two proteins in the two kinds of periodontal cells to assess the protein expression of the two genes (**Figure 2**). The MFI ratio for ICAM-1 or VCAM-1 in periodontal lymphocytes was significantly increased compared to that in normal lymphocytes (P<0.01), indicating that ICAM-1 or VCAM-1 was highly expressed in the periodontal lymphocytes.

Effects of antibody of target protein on periodontal lymphocytes apoptosis

The results showed that the percentage of apoptotic cells in periodontal lymphocytes treated with ICAM-1 antibody (about 15.0%) or VCAM-1 antibody (about 14.1%) was significantly increased compared to the control cells (P<0.05, **Figure 3**). Additionally, when cells were treated with the two kinds of antibodies, the percentage of apoptotic periodontal lymphocytes was further significantly increased (about 32.6%) than the controls (P<0.01). These results suggested that the high expression of ICAM-1 or VCAM-1 may be associated with the suppressed cell apoptosis during periodontitis.

ICAM-1 or VCAM suppression inhibited cell signal pathway-related protein expression

We further analyzed the cell apoptosis-related and NF-κB pathway-related protein expression in periodontal lymphocytes to investigate the potential molecular mechanism of ICAM-1 or VCAM expression on periodontal progression (**Figure 4**). The relative mRNA and protein levels for Bcl-2 and Bcl-xs were highly expressed by the treatment of ICAM-1 or VCAM antibody in periodontal lymphocytes, and this effect was more apparent when cells were treated with the

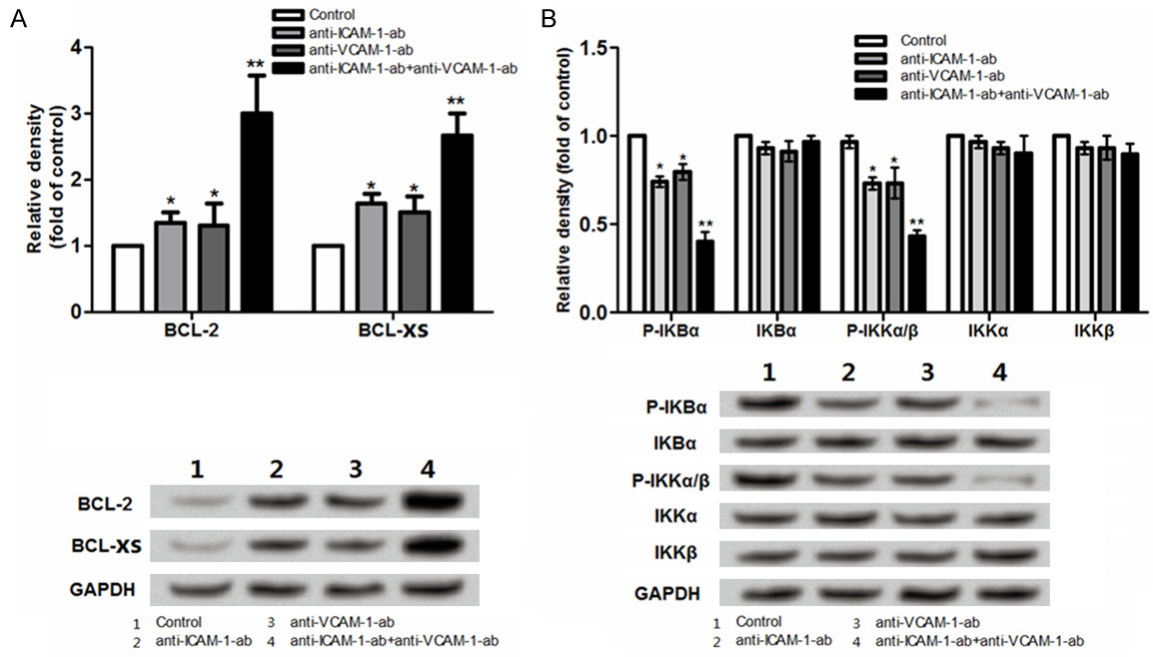

Figure 4. Effects of ICAM-1 and VCAM-1 antibody-treatment on cell signal pathway-related protein expression in periodontal lymphocytes. A: The relative mRNA and protein levels for Bcl-2 and Bcl-xs in ICAM-1 antibody- or VCAM-1 antibody-treated periodontal lymphocytes were significantly increased, and this effect was more apparent when cells treated with the two kinds of antibodies; B: The protein levels for the phosphorylated (p)-IKBα, p-IKKα/β, IKBα, IKKα and IKKβ were analyzed using western blotting. p-IKBα and p-IKKα/β levels were significantly decreased by the ICAM-1 antibody- or VCAM-1 antibody-treatment in periodontal lymphocytes, whereas their levels were drastically decreased when cells were co-treated with the two kinds of antibodies. *: P<0.05 and **: P<0.01 compared to the control (periodontal lymphocytes without any antibody treatment).

two kinds of antibodies (**Figure 4A**). Besides, when cells were treated with different kinds of antibodies, the phosphorylated (p)-IKBα and p-IKKα/β were both significantly decreased compared to that in control group (P<0.05), in addition, their levels were drastically decreased when periodontal lymphocytes were treated with the two kinds of antibodies (P<0.01).

Discussion

Periodontitis remains to be one of the most common infectious diseases, and brings huge causes to people's life [1, 22]. Previous evidence revealed the significant roles of the cell adhesion-related protein of ICAM-1 and VCAM-1 in the pathogenesis of periodontitis. However, few have mentioned their roles in the inflammatory biology of periodontitis. In this study, we investigated the expression of ICAM-1 and VCAM-1 in the inflamed gingiva-isolated periodontal lymphocytes and analyzed the effects of the two proteins in the development of periodontitis. In agreement with previous studies [16, 23], the relative mRNA expression of ICAM-1 and VCAM-1 was highly expressed in the periodontal lymphocytes (**Figure 1**). On the other side, flow cytometry assay showed that both ICAM-1 and VCAM-1 protein expression were up-regulated in periodontal lymphocytes (**Figure 2**), which was coincidence with their mRNA level. These results suggested that the abnormal expression of ICAM-1 and VCAM-1 may correlate to the pathogen of periodontitis.

Subsequently, we further analyzed the effects of ICAM-1 and VCAM-1 expression on the lymphocytes apoptosis by treating the cells with the according antibodies. Our results showed that the lymphocytes apoptosis was enhanced by the suppressed ICAM-1/VCAM-1 (**Figure 3**). The influence of ICAM-1/VCAM-1 expression on the apoptosis of periodontal lymphocytes has not been fully discussed. However, the roles of ICAM-1/VCAM-1 on cell apoptosis had been demonstrated in many kinds of diseases. Afford et al proved VCAM-1 promoted the inflammation by inhibiting the T cell apoptosis [24], and Yamagata et al demonstrated VCAM-1 was correlated to human endothelial cell apoptosis

[25]. In addition, Skowron et al proved that ICAM-1 was correlated to the apoptosis of human aortic smooth muscle cell [26]. Based on our data, we speculated that ICAM-1/VCAM-1 suppression may induce lymphocytes apoptosis during periodontitis development.

Meanwhile, we further investigated the potential molecular mechanism for the ICAM-1/VCAM-1 suppression on periodontitis progression. On one hand, the cell apoptosis promoter of Bcl-2 [27, 28] and Bcl-xs expressions [29] were both significantly enhanced by the suppressed ICAM-1/VCAM-1, and when the two kinds of antibodies were added into cells, the apoptosis-related protein was drastically enhanced (**Figure 4**), implying that apoptosis of periodontal lymphocytes may induced by the suppressed ICAM-1/VCAM-1 via increasing the Bcl-2 and Bcl-xs. From another point of view, cell signal transduction plays pivotal roles in the immune defense mechanism of host [30]. IKBα and IKKα/β are the enzyme complex that forms part of the NF-κB signaling pathway [31]. NF-κB signal pathway is involved in various biological processes including infection, inflammation, immune, cell apoptosis, and tumor pathogen, as well as cell cycle and differentiation [32, 33]. The activation of NF-κB including IKBα and IKKα/β could induce various factors transcription, such as cytokines, ICAM-1, and VCAM-1 [34]. In this study, when ICAM-1 and VCAM-1 were suppressed in periodontal lymphocytes, the levels of p-IKBα and p-IKKα/β were declined, and their levels were apparently decreased by the co-regulation of the two kinds of proteins, suggesting that the suppressed ICAM-1/VCAM-1 may play certain inhibit role in the inflammation by preventing the activation of NF-κB signal pathway.

In conclusion, the data presented in this study suggests that the up-regulated ICAM-1 and VCAM-1 is closely correlated to the pathogen of periodontitis through complicate mechanism. The overexpressed ICAM-1/VCAM-1 may contribute to the development and progression of periodontitis through inhibiting apoptosis and activating the NF-κB signal pathway. Our study may provide theoretical basis for the possible mechanism of ICAM-1/VCAM-1 in the pathogenesis of periodontitis and may reveal the potential application of ICAM-1/VCAM-1 in the genetic treatment of periodontitis. Further experimental studies are still needed to explore the deep mechanism at transcriptional level.

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This study was conducted at Department of Stomatology, Daqing Oil Field General Hospital.

Disclosure of conflict of interest

None.

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References

[1] Procházková L and Souček M. Oral infection and rheumatic diseases. Vnitr Lek 2014; 60: 128-133.

[2] Pfirrmann RW and Geistlich P. Treatment of dentoalveolar infections with taurolidine and/or taurultam. US, US 6488912 B1. 2002.

[3] Laxman VK and Sridhar A. Tobacco use and its effects on the periodontium and periodontal therapy. J Contemp Dent Pract 2008; 9: 97-107.

[4] Piramal SA, Jathar SR, Sirwani RP and Piramal SA. Herbal compositions for the treatment of diseases of the oral cavity: US, US 8182843 B2. 2012.

[5] Fabri GMC, Sannomiya P, Prado CM, Leick-Maldonado EA, De Siqueira SRDT, Tibério IFC, Negri EM, Bizeto L, Cruz JWM and Siqueira JT. The expression of nitric oxide in the gingival tissue in subjects with periodontitis and chronic pain. International Journal of Odontostomatology 2014; 8: 279-287.

[6] Nair S, Faizuddin M and Dharmapalan J. Role of autoimmune responses in periodontal disease. Autoimmune Dis 2014; 2014: 596824-596824.

[7] Chapper A, Munch A, Schermann C, Piacentini CC and Fasolo MTM. Obesity and periodontal disease in diabetic pregnant women Obesidade e doença periodontal em gestantes diabéticas. Braz Oral Res 2005; 19: 83-87.

[8] Ardila Medina CM, Ariza Garcés AA and Guzmán Zuluaga IC. Coexistencia de porphyromonas gingivalis, tannerella forsythia y treponema denticola en el complejo rojo bacteriano en sujetos co periodontitis crónica. International Journal of Odontostomatology 2014; 8: 359-364.

[9] Lucas H, Bartold PM, Dharmapatni AA, Holding CA and Haynes DR. Inhibition of apoptosis in periodontitis. J Dent Res 2010; 89: 29-33.

[10] Wyllie AH. "Where, O death, is thy sting"? A brief review of apoptosis biology. Mol Neurobiol 2010; 42: 4-9.

[11] Vaux DL and Strasser A. The molecular biology of apoptosis. Proc Natl Acad Sci U S A 1996; 93: 2239-2244.
[12] Gehlsen KR. Method and compositions for topical treatment of damaged tissue using reactive oxygen metabolite production or release inhibitors: US, US 6270781 B1. 2001.
[13] Wang N, Verna LS, Forsayeth J, Zhu Y and Stemerman M. Adenovirus-mediated overexpression of c-Jun and c-Fos induces intercellular adhesion molecule-1 and monocyte chemoattractant protein-1 in human endothelial cells. Arterioscler Thromb Vasc Biol 1999; 19: 2078-2084.
[14] Ishikawa T, Imura A, Tanaka K, Shirane H, Okuma M and Uchiyama T. E-selectin and vascular cell adhesion molecule-1 mediate adult T-cell leukemia cell adhesion to endothelial cells. Blood 1993; 82: 1590-1598.
[15] Molé N, March KD, Martin G, Miller N, Béné MC and Faure GC. High levels of soluble intercellular adhesion molecule-1 (ICAM-1) in crevicular fluid of periodontitis patients with plaque. J Clin Periodontol 1998; 25: 754-758.
[16] Rezavi K, Palmer RM, Odell EW, Scott DA and Wilson RF. Expression of ICAM-1 and E-selectin in gingival tissues of smokers and non-smokers with periodontitis. J Oral Pathol Med 2002; 31: 59-64.
[17] Tsurumachi T, Nishi K, Hayashi M, Saito T, Saito I and Moro I. VLA-4/VCAM-1 pathway in human periodontal disease. Adv Exp Med Biol 1995; 371b: 1131-1133.
[18] Naoko O, Kazumi F, Yoji T, Murat D, Kazuo T, Hiroshi F, Kenji M, Toshiharu N and Hirohisa S. The implications of the upregulation of ICAM-1/VCAM-1 expression of corneal fibroblasts on the pathogenesis of allergic keratopathy. Invest Ophthalmol Vis Sci 2005; 46: 4512-4518.
[19] Sawa T, Nishimura F, Ohyama H, Takahashi K, Takashiba S and Murayama Y. In vitro induction of activation-induced cell death in lymphocytes from chronic periodontal lesions by exogenous Fas ligand. Infect Immun 1999; 67: 1450-1454.
[20] Yamamoto H, Okamoto Y, Horiguchi S, Kunii N, Yonekura S and Nakayama T. Detection of natural killer T cells in the sinus mucosa from asthmatics with chronic sinusitis. Allergy 2008; 62: 1451-1455.
[21] Siqueira M, Fischer R, Pereira N, Martins M, Moss M, Mendes-Ribeiro A, Figueredo C and Brunini T. Effects of non-surgical periodontal treatment on the L-arginine-nitric oxide pathway and oxidative status in platelets. Exp Biol Med (Maywood) 2013; 238: 713-722.
[22] Karlsson E, Lymer UB and Hakeberg M. Periodontitis from the patient's perspective, a qualitative study. Int J Dent Hyg 2009; 7: 23-30.
[23] Liu YS, Liang BI, Lin J, Dai JY, Xin SU and Jia BI. Expression of MCP-1 and ICAM-1 in periodontal fiber in a rat model with experimental periodontitis. Chinese Journal of Conservative Dentistry 2013.
[24] Afford SC, Humphreys EH, Reid DT, Russell CL, Banz VM, Ye O, Vo T, Jenne C, Adams DH and Eksteen B. VCAM-1 expression by biliary epithelium promotes persistence of inflammation by inhibiting effector T cell apoptosis. Hepatology 2013; 59: 1932-1943.
[25] Yamagata K, Xie Y, Suzuki S and Tagami M. Epigallocatechin-3-gallate inhibits VCAM-1 expression and apoptosis induction associated with LC3 expressions in TNFα-stimulated human endothelial cells. Phytomedicine 2015; 22: 431-437.
[26] Skowron W, Zemanek K, Wojdan K, Gorzelak P, Borowiec M, Broncel M and Chalubinski M. The effect of interleukin-35 on the integrity, ICAM-1 expression and apoptosis of human aortic smooth muscle cells. Pharmacol Rep 2014; 67: 376-381.
[27] Lê MG, Marie-Christine M, Setha DR, Le BML, Howayda AEA, Marc S and Guy R. c-myc, p53 and bcl-2, apoptosis-related genes in infiltrating breast carcinomas: Evidence of a link between bcl-2 protein over-expression and a lower risk of metastasis and death in operable patients. Int J Cancer 1999; 84: 562-567.
[28] Laulier C and Lopez BS. The secret life of Bcl-2: apoptosis-independent inhibition of DNA repair by Bcl-2 family members. Mutat Res 2012; 751: 247-257.
[29] Finucane DM, Bossy-Wetzel E, Waterhouse NJ, Cotter TG and Green DR. Bax-induced caspase activation and apoptosis via cytochrome c release from mitochondria is inhibitable by Bcl-xL. J Biol Chem 1999; 274: 2225-2233.
[30] Briken V, Miller JL. Living on the edge: Inhibition of host cell apoptosis by Mycobacterium tuberculosis. Future Microbiol 2008; 3: 415-422.
[31] Tian F, Zhou P, Kang W, Luo L, Fan X, Yan J and Liang H. The small-molecule inhibitor selectivity between IKKα and IKKβ kinases in NF-κB signaling pathway. J Recept Signal Transduct Res 2015;35:307-18.
[32] Aradhya S and Nelson DL. NF-κB signaling and human disease. Curr Opin Genet Dev 2001; 11: 300-306.
[33] Fraser CC. Exploring the positive and negative consequences of NF-κB inhibition for the treatment of human disease. Cell Cycle 2006; 5: 1160-1163.
[34] Cui J, Chen PB, Yang XF and Huang H. [Effect of the simple acupoint catgut embedding on the expression of ICAM-1, NF-κB and airway inflammation in rats with asthma]. Zhongguo Zhen Jiu 2010; 30: 141-5.